

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		ATTORNEY'S DOCKET NUMBER 5625 U.S. APPLICATION NO. <div style="font-size: 1.5em; font-weight: bold;">10/031365</div>
INTERNATIONAL APPLICATION NO. PCT/GB00/02801	INTERNATIONAL FILING DATE 20 July 2000	PRIORITY DATE CLAIMED 20 July 1999
TITLE OF INVENTION ELECTRODES FOR GENERATING AND ANALYZING DIELECTROPHORESIS		
APPLICANT(S) FOR DO/EO/US Gary LOCK; and Ronald PETHIG		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. <input checked="" type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> has been communicated by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input checked="" type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (Unexecuted) 10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p style="margin-left: 20px;">Items 11 to 20 below concern document(s) or information included:</p> <ol style="list-style-type: none"> 11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input type="checkbox"/> A FIRST preliminary amendment. 14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 20. <input checked="" type="checkbox"/> Other items of information: <ul style="list-style-type: none"> - Application Data Sheet - Amended claims 1-31 - Statement Under Article 19 		

U.S. APPLICATION NO.

10/031365

INTERNATIONAL APPLICATION NO.
PCT/GB00/02801

ATTORNEY'S DOCKET NO.
5625

21. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482)
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO
and International Search Report not prepared by the EPO or JPO.....\$1,040

International preliminary examination fee (37 CFR 1.482) not paid to
USPTO but International Search Report prepared by the EPO or JPO.....\$ 890

International preliminary examination fee (37 CFR 1.482) not paid to
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International preliminary examination fee (37 CFR 1.482) paid to USPTO
but all claims did not satisfy provisions of PCT Article 33(1)-(4).....\$ 710

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CALCULATIONS FOR PTO USE ONLY

\$ 890.00

Surcharge of \$130.00 for furnishing the oath or declaration later than
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☐ 20 ☒ 30

\$ 130.00

CLAIMS

NUMBER FILED

NUMBER EXTRA

RATE

\$

Total claims

47 - 20 =

27

x \$ 18.00

\$ 486.00

Independent claims

8 - 3 =

5

x \$ 84.00

\$ 420.00

MULTIPLE DEPENDENT CLAIM(S) (if applicable)

+\$280.00

\$ 280.00

TOTAL OF ABOVE CALCULATIONS =

\$2,206.00

☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above
are reduced by 1/2.

\$ 0.00

SUBTOTAL =

\$2,206.00

☐ Processing fee of \$130 for furnishing the English translation later than
months from the earliest claimed priority date (37 CFR 1.492(f)).

☐ 20 ☐ 30

\$ 0.00

TOTAL NATIONAL FEE =

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Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by
an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property

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TOTAL FEES ENCLOSED =

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a. ☒ A check in the amount of \$ 2,206.00 to cover the above fees is enclosed.

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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be
filed and granted to restore the application to pending status.

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33.161

REGISTRATION NUMBER

Date: January 18, 2002

g/pst

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ELECTRODES FOR GENERATING AND ANALYSING DIELECTROPHORESIS

This invention relates to an apparatus for analysing particles, especially by the technique of
5 dielectrophoresis (DEP) or travelling wave dielectrophoresis (TWD) or electrorotation and relates particularly to a device for monitoring or controlling the DEP or TWD or electrorotation process; in this specification the three types of process will be
10 referred to as AC electrokinetic processes or effects.

Throughout this specification, the term "particle" is used to include biological cells, bacteria, viruses, parasitic microorganisms, DNA, proteins, biopolymers,
15 non-biological particles, or any other particle which may be suspended in a liquid, in which an AC electrokinetic force can be induced. It also applies to chemical compounds or gases dissolved or suspended in a liquid, where an AC electrokinetic force can be induced.

20

The technique of dielectrophoresis is well-known and is described in the book "Nanotechnology in Medicine and the Biosciences", edited by R R Combs and D W Robinson, published by Gordon and Breach, Amsterdam, in chapter 11

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by Ronald Pethig, especially pages 88 to 95.

Dielectrophoresis is the movement of particles in non-uniform electric fields. Unlike electrophoresis, charges on the particle itself are not necessary for the effect to occur, and AC rather than DC fields are employed.

The technique of using DEP in combination with Field Flow Fractionation is described in the publication "DEP-FFF: Field-Flow Fractionation using Non-uniform Electric Fields" by G H Markx, J Rousselet and R Pethig, published in 1997 in the Journal of Liquid Chromatography and Related Technologies, volume 20, pages 2857-2872. This publication describes how the flow velocity of the particle suspending fluid strongly influences the DEP force acting on a particle. The use of TWD for manipulating cells is described in the publication "Electromanipulation and Separation of Cells using Travelling Electric Fields" by M S Talary, J P H Burt, J A Tame and R Pethig, published in 1996 in the Journal of Physics D: Applied Physics, volume 29, pages 2198-2203. This publication describes how the DEP force acting on a biological cell can be determined by measuring the speed and direction of cell motion.

Of relevance to this present invention are the factors that control the DEP force acting on a particle suspended in a fluid medium. For a spherical particle of radius r suspended in a medium of absolute dielectric permittivity ϵ_m the DEP force is given by:

$$F_{DEP} = 2 \pi r^3 \epsilon_m \alpha (\nabla E^2)$$

In this equation α is a parameter (the real part of the Clausius-Mossotti factor) that defines the effective polarisability of the particle with respect to the

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suspending fluid medium and the factor (∇E^2) quantifies the gradient and strength of the electric field E acting on the particle. Depending on such factors, as the conductivity, permittivity, temperature, pH, chemical content of the suspending fluid medium, the polarisability parameter α can theoretically have a value that ranges between +1.0 and -0.5. In order to control the DEP force, all of these factors need to be measured and controlled. The DEP force is also influenced by the fluid flow velocity, and therefore fluid viscosity. For a given AC voltage applied to the set or plurality of electrodes, the electric field and field gradient depends on the geometry and physical integrity of the electrodes. The field strength and gradient can change if, during the DEP process (or after cleaning the electrodes between DEP processes), the electrodes deteriorate. The forms of deterioration include the cases where adhesion of the metal electrodes to their substrate becomes impaired: the width, thickness or shape of individual electrode elements changes, the distance between adjacent electrodes changes: particles or other solid or chemical matter becomes permanently associated with (e.g. clogs) the electrodes. In the case of TWD processes, the operating conditions can also be monitored indirectly by measuring the induced movement of reference or model particles.

An effect that is closely related to DEP is that of Electrorotation. This is the effect whereby a particle is induced to rotate in a rotating electric field. The rotational torque exerted on a particle in a rotating electric field is proportional to the imaginary part of the Clausius-Mossotti factor describing the parameter α in the DEP force equation given above. The Electrorotation effect and its relationship to DEP is described in the publication entitled "Differences in

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the AC Electrodynamics of Viable and Non-viable Yeast Cells determined through combined Dielectrophoresis and Electrorotation Studies" by Y Huang, R Hozel, R Pethig and X B Wang, published in 1992 in the Journal of
5 Physics in Medicine and Biology, vol. 37, pages 1499-1517.

In this invention, a device is described that enables continuous electrical monitoring and controlling of the
10 various factors described above, any one of which can have a major effect on AC electrokinetic processes.

Depending on the applied frequency and the precise arrangement, the AC electrokinetic effect is highly
15 dependent on pH, on electrical conductivity and temperature, or on permittivity. Small variations in such a variable can have a major effect on the DEP process.

20 In a publication in Electronics Letters 8 January 1998, vol. 34, No 1, pages 66, Milner et al disclose a dual-channel electrode structure in which matching pairs of electrodes are respectively exposed to a bacteria suspension and water and impedance changes are sensed to
25 monitor the bacteria collection between the electrodes, but there is no monitoring of the DEP process itself or of electrode characteristics. This is an alternative to the well-known optical techniques.

30 WO 95/17258 describes a method for manipulating particles using dielectrophoresis, and a method for using chemicals whose fluorescence is pH-sensitive to show visually the creation of a pH gradient in a DEP cell, but does not disclose any probe arrangement for
35 actually monitoring pH in the cell.

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According to the invention, an electrode formation for use in a dielectrophoretic cell comprises a substrate, and, on the substrate

- 5 a set of a plurality of electrodes arranged for the application of an AC electrokinetic force; and
- a measurement electrode array arranged for the measurement of an AC electrokinetic-related
- 10 parameter.

Preferably the substrate is of a non-conducting material such as glass or rigid plastics material. The substrate may be a microscope slide, and the electrodes may be

15 applied to the slide by the technique of photolithography, or other semiconductor or microfabrication technique, such as use of an excimer laser.

20 Preferably the measurement electrode array is connectable to means to measure the permittivity or the electrical conductivity or the impedance of any liquid in contact with those electrodes, or to measure the pH or the temperature or the flow rate of a fluid in

25 contact with or adjacent those electrodes. From the measurements the presence or passage of particles or the speed of particles or the fluid viscosity may be determined.

30 For the measurement of temperature, the measurement electrode array may be a long electrode arranged in the form of a double coil or compacted sinusoid. For the measurement of permittivity or electrical conductivity, the measurement electrode array may be in the form of

35 spaced parallel electrodes. For the measurement of pH, the measurement array may comprise two electrodes, the

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surface of the electrodes carrying a thin film of inorganic salt, so as to form a solid state pH electrode.

- 5 Alternatively, the electrodes may have silicon between them. Similarly chemical content may also be measured by using a thin film of an appropriate material.

10 Optionally there is provided a second set of electrodes for measuring the electrical conductivity and/or permittivity and a third set of electrodes for measuring temperature.

15 For detection of the presence or passage of particles, the measurement electrode array comprises a pair of pointed electrodes spaced across the direction of movement of the particle. For measurement of particle speed, two or more pairs of such electrodes are provided, the pairs being spaced along the direction of movement of a particle. For detection of fluid flow, 20 the measurement electrode array comprises a thin electrode transverse to the direction of flow, there further being provided means to provide a heating current to the thin electrode. Preferably there are several such arrays provided, spaced along and/or across 25 the direction of flow.

A great advantage of an electrode formation according to the invention is that the set of electrodes measuring 30 the AC electrokinetic-related parameter can be located in very close proximity to the electrodes. In previous arrangements, measurements of electrical conductivity and/or temperature of the liquid to which the DEP field was applied were made at positions distant from the DEP 35 electrodes, thus introducing considerable uncertainty and error to the DEP process and experimental

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conditions.

Arnold et al, J. Phys. Chem, 91, pages 5093-5098, 1987,
describe a method for monitoring the conductivity of the
5 suspending medium during electrorotation measurements
that employs the electrodes used to generate the
rotating electric field. This method has the
disadvantage, overcome by the present invention, that
the conductivity cannot be monitored at the same time as
10 the electrorotation measurements. Arnold et al in the
same paper describe the use of a conventional
thermocouple, composed of macroscopic wires for
monitoring temperature. In this invention, the
temperature probe takes the form of a resistive element
15 fabricated by photolithography directly on to the
substrate containing the electrode arrays.

Hölzel, Biophysical Journal 73, pages 1103-1109, 1997,
describes the use of platinum wires for monitoring the
20 medium conductivity during electrorotation measurements.
In this invention, microscopic electrodes are used for
conductivity determination and are fabricated by
photolithography to be in close proximity to the
electrode arrays.

25 The substrate may be integral, for example a single
glass slide or the like, or divided, e.g. two or more
contiguous glass slides.

30 The invention will now be described by way of example
only with reference to the accompanying drawings in
which:

Figure 1a illustrates an electrode formation according
35 to the invention;

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Figures 1b and 1c are details of parts of Figure 1;

Figure 2 provides further details of a DEP cell;

5 Figure 3 illustrates a DEP monitoring system;

Figures 4 and 5 illustrate alternative measuring electrode arrangements;

10 Figure 6 illustrates an alternative TWD monitoring system;

Figure 7 illustrates a variation of Figure 1;

15 Figure 8 illustrates an electrorotation electrode array;

Figure 9 shows a system for controlling a sensed AC electrokinetic parameter;

20 Figure 10 shows a connection system for the array of Figure 1a;

Figure 11 illustrates electrode defects;

25 Figure 12 shows an electrode parameter sensing system;

Figures 13 and 14 illustrate further electrode defects; and

30 Figure 15 shows an alternative electrode parameter sensing system;

Figure 16 shows a temperature calibration curve for the probe shown in Figure 1b;

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Figure 17 shows a conductivity calibration curve for the

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probe shown in Figure 1c.

In Figure 1a, a glass substrate 10 carries three sets of electrodes, all fabricated by the technique of photolithography and comprising a layer of chromium covered by a layer of gold. The first set comprises a pair 12, 14 of parallel electrodes each connected to an edge connection 16, 18 at the edge of the substrate. The pair 12, 14 can be used to measure the conductivity of and the permittivity of any liquid or suspension overlying them. The set is shown in more detail in Figure 1c.

A second set comprises a multiplicity of straight, parallel electrodes forming an array 20, each electrode being connected to one of an array of edge connections 22, 24 lying along opposite sides of the substrate 10. The electrodes in the array 20 are straight, parallel, travelling wave (TWD) electrodes as are well known in DEP technology and may apply a TWD field to any liquid or suspension overlying them. Alternatively, the electrodes may be of castellated form, or arranged to apply a rotating field, as is well-known.

A third set comprises a single, long, thin electrode arranged in the shape of a double coil 24, connected to a pair of connections 26, 28 at the edge of the substrate. The coil can be used to measure the temperature of any liquid or suspension or air or other material in its vicinity. The coil is shown in more detail in Figure 1b.

A calibration curve for the temperature probe arrangement of Figure 1b is shown in Figure 16 which plots the resistance R of the probe measured with a standard digital multimeter, against temperature T. It

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can be seen that temperature variations to within $\pm 0.1^\circ\text{C}$ can be detected over a working temperature range from 4°C to 60°C . A temperature probe having the characteristic shown in Figure 16 has been used to

5 monitor temperature changes occurring in a DEP cell separation chamber to monitor temperature changes arising from, for example, external light illumination, fluid flow, electrical energisation of the electrodes and temperature variations of the external environment.

10 A calibration curve for the conductivity probe arrangement of Figure 1c is shown in Figure 17, which shows probe impedance Z as a function of the conductivity C of the medium. Using this calibration

15 curve, the conductivity of the suspending medium can be determined to within ± 2 mS/m for the range 50 mS/m to 200 mS/m, and to within ± 0.5 mS/m for the range 10 mS/m to 50 mS/m.

20 Figure 2 shows a substrate 10 and three sets of electrodes as in Figure 1a, but shows also an oval spacer 38 which is placed on the substrate so as to surround all three sets of electrodes and to overlie the connections. Figure 3 shows that a second substrate 36

25 is placed on top of the spacer 38 to enclose a volume in which a suspension of particles can be contained for analysis by TWD techniques. Figure 3 also shows schematically that the electrodes 12, 14 are connected to an electrical conductivity measurement device 30,

30 such as an AC bridge operating at a frequency greater than 50 kHz to avoid problems of electrode polarisation. The double coil 24 is connected to a temperature measurement device 32 such as a DC bridge. A TWD electrode array 20 is connected to a frequency generator

35 34.

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The volume within the spacer 38 is illuminated from below by a light source 40 and viewed by a microscope/video camera 42 connected to a display screen 44 on which movement of particles under the influence of the TWD field applied by electrode array 20 can be monitored. The electrical conductivity and the temperature of the liquid in which the TWD effect is taking place are monitored by the devices 30 and 32 respectively.

The close proximity of the three sets of electrodes allows accuracy of TWD process control to a level which has not previously been achievable.

This is especially important for biological samples because the conductivities of aqueous electrolytes and biological fluids are sensitive to changes in temperature, a typical change being of the order of 2-3% increase in conductivity per degree Centigrade increase in temperature.

Further, mammalian cells can tolerate considerable drops in temperature, but they cannot tolerate more than about 2°C above the normal physiological temperature of 37.5°C for more than a few hours, and will die rapidly at 40°C and over (R I Freshney, Culture of Animal Cells, 3rd Ed. Wiley-Liss, New York, page 102, 1994).

While Figure 1 illustrates an array 20 of linear electrodes suitable for the application of a TWD field, it may be replaced by a castellated array. Alternatively, the array may be electrodes suitable for the application of a static DEP or electrorotational field.

While the example described with reference to Figure 3

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describes a static, non-flow system, the simple addition of input and outlet connections and pipes allows the electrode formation according to the invention to be used in a throughflow, conventional DEP system, in which
5 case the array 20 is arranged for the application of a conventional DEP field. In addition, a gravity flow system could be provided by turning the substrates 10, 36 to form a vertical cell.

10 In addition to measurement of temperature and conductivity, other properties can be sensed, such as fluid flow and particle movement.

Figure 4 shows a probe which can measure the flow of the
15 particle suspension. The probe comprises a thin wire 50 between connecting pads 46, 48. If a current is applied to heat the wire, liquid flow over it will cause a cooling effect. Flow rate can be determined by
conventional techniques of either varying the applied
20 energy to keep the temperature of the wire constant, or applying constant energy and measuring the temperature of the wire 50.

Usually a cell operating with fluid flow will be
25 provided with several fluid flow probes across the direction of flow. While the fluid flow rates generally vary in a parabolic profile across the cell, variations from the theoretical profile may be caused by viscosity variations and surface properties of the cell
30 substrates. Measurement of the actual flow rates at several positions is therefore valuable. In a variation, several fluid flow probes may be provided spaced along the direction of flow.

35 Figure 5 shows a TWD arrangement with particle detection. An array 58 of TWD electrodes is connected

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so that the field travels from left to right as shown by the arrow 68. Spaced along the array are a first pair 54 and a second pair 56 of particle detection electrodes, each electrode having a pointed end adjacent the array 58. Each pair is connected to an impedance sensing device (not shown). When a suspended particle passes between the pair of electrodes 54, a change of impedance occurs, and therefore the presence of the particle can be sensed, and a particle count can be provided. Also flow blockages or particle sticking can be sensed.

When the same particle passes between the pair of electrodes 56, another change of impedance occurs, and from the knowledge of the distance between the electrode pairs 54, 56, particle speed can be determined.

By more complex measurements, particle levitation height above the TWD electrodes and particle size can also be measured. Either of the arrangements of Figures 4 and 5 can also be used to sense a cessation of flow of particles, which might indicate a blockage at an upstream point of the system. Also by passage of known particles, the arrangements can be used to measure the viscosity of the suspending medium.

When the presence and/or speed of travel of a particle can be sensed, the TWD process can be automated, as shown in Figure 6. The left hand side is identical to the Figure 5 arrangement, but in addition there are two further sets of TWD electrodes 60, 62, which overlap in the vicinity of the end of the array 58, but then diverge to form two separate arrays. Only one of the further sets 60, 62 will be energised at a time, and the arrangement forms a switched junction.

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- In use, the speed of each particle passing along the array 58 is sensed by the probe pairs 54, 56; the speed characterises the particle so that different particles in a mixture can be identified. As each particle reaches the right hand end of the array 58, one of the electrode arrays 60, 62 is energised and the particle moves along the energised array. Particle separation can therefore be achieved.
- 10 In all arrangements, the liquid to be measured may be in direct contact with the electrodes; for some measurements, such as pH, fluid flow or temperature measurements, the electrodes may be covered by a thin film layer.
- 15 In a variation (not illustrated), the pH of a fluid may be measured by a pair of spaced electrodes having between them a piece of silicon. Alternatively, the surface of one electrode may be covered by a thin layer of inorganic salt, so as to form a solid state pH electrode. The electrochemical potential between the two electrodes can then be measured to indicate pH. By the application of different thin film layers to the electrodes, selectivity can be achieved as different types of layer have different sensitivity to different ions. Similarly, films can be chosen to sense specific chemical contact of the solution and determine factors such as anti-body levels.
- 30 Another way to provide a chemical pH probe is by the use of an ISFET (ion sensitive field effect transistor) comprising a MOSFET fabricated on a piece of silicon, covered with a thin film of a polymer such as Si_3N_4 , Al_2O_3 , and placed between a set of electrodes. The presence of ions affects the transistor, allowing pH to be measured.

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In another variation (not illustrated), the AC electrokinetic-related parameter to be sensed is chemical composition; this can be sensed by selective application of a protein, such as an antibody, or of the combination of a protein and a protein-binding ligand, such as biotin or avidin. It is also possible to use the selective application of an oligonucleotide for hybridisation to an RNA or DNA sequence.

- 10 Accurate control of pH is especially important for maintaining cell viability when pH is best kept between 7.4 ± 0.2 particularly for mammalian cells. Figure 7 shows an array 70 of interdigitated DEP electrodes, a double coil temperature probe 72 connected to a pair of connections 74, 76 and a conductivity probe comprising a pair of spaced linear electrodes 78, 80 connected to respective connections 82, 84. The conductivity probe is placed between the inter-digitated electrode.
- 15
- 20 The arrangement is prepared by fabricating the temperature probe 72 on a substrate, applying an insulating layer, such as a layer of polyimide, over the temperature probe, and then fabricating the interdigitated array and the conductivity probe on top of the polyimide layer.
- 25

With such an arrangement, the temperature probe 72 measures the temperature of the suspension which is in contact with the interdigitated array 70, and accuracy is improved.

30

Since it is important to have the smallest possible distance between the electrodes generating the AC electrokinetic effect and the electrodes measuring the conductivity and/or permittivity of the suspending fluid, in the extreme case the generating electrodes

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themselves can be used as the measuring electrodes. For example, in Figure 1a, the first set of electrodes 12, 14 and the double coil electrode 24 can simply be omitted, and the second set of electrodes forming the array 20 can be connected to sensing means to measure conductivity and/or permittivity. The electrode array 20 can also be connected to sensing means to perform a self-test of the electrical and mechanical integrity of the array 20.

10

The invention can also be applied to electrorotation, and Figure 8 indicates schematically an electrical probe for such an application. Four electrodes 86 are arranged along radii on a circular substrate 88, the radii being separated by 90°. The electrodes 86 are spaced around a centrally-arranged double coil temperature-sensing electrode 90 connected to two connections 92. In use, signals at relative phases of 0°, 90°, 180° and 270° are applied to the electrodes 86 to create an electrorotation field. The construction may be similar to that described with reference to Figure 7, i.e. the temperature-sensing electrode 90 is deposited on the substrate 88, covered with an insulating film, and the electrorotation electrodes 86 are deposited on top of the film. This prevents the temperature-sensing electrode 90 from affecting the electrorotation field.

The invention has been described in arrangements for monitoring AC electrokinetic parameters; it can also be used to control such parameters. A general experimental arrangement is shown in Figure 9, which is a variation of part of Figure 3, and identical integers are given identical reference numerals. Figure 9 additionally illustrates a Peltier cooling device 94 bonded to the outer surface of the lower substrate 10 just below the

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temperature coil. The temperature coil on the substrate 10 is connected to a temperature measurement and control device 32A which, in this variation, is also connected to the Peltier device 94. The temperature control device 32A stores a required operating temperature of the fluid in the cell; when the measured temperature increases, the device changes the current flow to the Peltier device 94 which causes it to apply cooling until the required temperature is regained.

Such an arrangement is useful in, for example, a cell arranged to separate red blood cells and cancer cells when careful temperature control is vital. The electrode array 20 may also be a TWD array.

The inventive electrode array allows much greater accuracy of measurement of AC electrokinetic-related parameters than has previously been possible, and it is highly advantageous if the monitoring circuitry is positioned as close as possible to the electrode array.

In a variation of Figure 9, external fluid flow in conjunction with temperature probes may be used to control the temperature of the AC electrokinetic cell.

The fluid may flow in channels machined in the substrate, or applied external to the substrate.

A further variation of Figure 9, where an electrode array is used to act as a heating element, either fabricated on the inner walls of the substrate, within the cell, or on the outer walls. Used in conjunction with the temperature measuring means, this allows for the controlling of temperature.

Alternatively external air flow may also be used for the controlling of temperature.

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Any of the variants may be used in conjunction. The peltier cooler is particularly effective in that when supplied with a suitable electric current, it may be used for both heating and cooling.

5

Fluid flow may also be controlled using the electrode configuration of Figure 4 in conjunction with a hydrodynamic pump. Implemented with a similar configuration to that of Figure 9.

10

Figure 10 illustrates an arrangement by which this can be achieved. A printed circuit board 130 has a centrally-mounted electrical test socket 132, such as a modified 84-way PLCC socket with spring-loaded gold connections. The socket carries a number of electrical connections 134 arranged in hollow square. A substrate 10 is indicated in outline; reference to Figure 1A will show that the substrate has a number of electrical connections 22 also arranged in a hollow square which match the connections 134 when the substrate 10 is placed face-down on the test socket 132, thus making an electrical connection.

15

20

The PCB 130 also carries instrumentation 136, 138 for measuring conductivity and temperature; connection means 140 for connection to other instrumentation; and connection means 142 for connection of the four phase signals to the TWD electrode array on the substrate 10.

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The close proximity of the means for measuring conductivity and temperature to the electrode array on substrate 10, with no need for interconnecting wires, further improves the accuracy of the measurement.

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In application, the controlling of AC electrokinetic parameters will most commonly take the following forms:

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- i) Measuring and controlling of particle suspension conditions for the optimal conditions to preserve particles integrity and viability.
- 5 ii) Measuring and controlling of particle suspension conditions for conditions which favourably alter the AC electrokinetic behaviour to particle separation or differentiation advantage.
- 10 iii) Accurate controlling of fluid flow to benefit particle separation efficiency, from a combined hydrodynamic and dielectrophoretic separation.

Other common applications include the monitoring of
15 specific chemical content for the accurate sensing and control of antibody dispensing to particles within an AC electrokinetic cell. Accurate temperature control is also critical in this case due to the temperature sensitive nature of antibodies. Control of other AC
20 electrokinetic parameters is also beneficial.

In general, both temperature and pH must be closely controlled for living cells. For example, mammalian cells must be kept at 37°C and pH 7.4 for ensured
25 viability. Other types of particle, such as proteins, require a temperature of 4°C. Yet other particle types, such as bacteria, will have other preferred temperatures and may prefer slightly acidic conditions.

30 Another application of control of the AC electrokinetic-related parameter is that separation of the types of particle can be enhanced. For example, temperature control can be used to separate the types of bacteria, e.g. campylobacter and gram negative bacteria by raising
35 the temperature of the suspension so that one type of bacteria is killed while the other remains viable.

- 20 -

Elevation of temperature or change of pH can also be used to separate two proteins by causing one to denature while the other remains globular. Very precise control of temperature and/or pH is necessary to achieve this,
5 as provided by the current invention.

The inventive arrangement can also be used to monitor the AC electrokinetic electrodes themselves. AC electrokinetic phenomena are highly dependent on
10 electrical field and on electrical field gradient, which are dependent upon electrode defects. Such defects may occur during fabrication, may be caused during cleaning processes, or may result from degradation during use.

15 Figure 11 illustrates an array of serpentine TWD electrodes 100 (as disclosed in our co-pending Application 99168509.2 filed on even date). In two areas 102, shown circled, there are fabrication defects which may be sufficiently severe to cause adjacent
20 electrodes to be in electrical connection. Such a fault can be detected relatively easily by simply measuring the electrical DC impedance between adjacent electrodes, but for cases where there is no electrical shorting, detection is less easy.

25 In use, the array of electrodes 100 will be connected to signals at phases of 0° , 90° , 180° and 270° , the phase pattern being repeated along the array. From the well-known principles of electrical filter and network
30 theory, the electrodes can be considered as distributed RC or RG, where R is resistance, C is capacitance and G is conductance.

In circumstances in which the conductivity dominates,
35 short lengths of adjacent electrodes 100 can be represented by an RG section. In circumstances in which

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the permittivity, and therefore the capacitance, dominates, short lengths of adjacent electrodes 100 can be represented by sections of series RC.

5 Thus, any electrode imperfection will significantly affect the impedance between adjacent electrodes, and therefore between "channels", i.e. all electrodes connected to the same phase; the effect is frequency dependent.

10

By measuring the impedance between different channels over the whole range of frequencies to be applied to the electrode array during use in the AC electrokinetic process, electrode integrity can be determined.

15

A suitable circuit for such a measurement is shown in Figure 12. The four phase signals 0° , 90° , 180° , 270° are each connected through a respective buffer amplifier 104 ABCD and isolating resistor 106 ABCD to an

20 electrical connection 108 ABCD. The connections 108 are connected to respective channels 110, 112, 114, 116 which represent the electrodes 100 to which the signals at different phases are connected so as to generate TWD conditions. Typically the frequency range to be applied
25 will be 10 kHz to 10 MHz.

The signal-generating circuitry and impedance measurement circuitry are referenced 118 and are arranged to measure the voltages between the output of
30 the buffer amplifiers 104 and the respective resistors 106, and between the resistors and the electrodes at the electrical connections 108. The connections 120, 122 for measurement at phase 0° are indicated and are identical for other phases.

35

The isolating resistors 106 and the impedance of the

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electrodes in respective channels form an electrical potential divider network, and the impedance of each of the four channels at each applied frequency can be determined. Typically tests can be carried out at a
5 range of frequencies, usually from low to high value; at some frequency, a substantial change in voltage will occur at the connection point 108.

In an example, the electrodes as shown in Figure 11 were
10 8 microns wide with an inter electrode gap of 8 microns at the narrowest point and 17 microns at the widest point and were in contact with a medium of conductivity $100\mu\text{S}/\text{cm}$, the medium comprising 320 mM sucrose, 3 g/l glucose, $1\mu\text{M}$ CaCl_2 . A signal of two volts peak to peak
15 was applied to each of the channels 110 to 116 and the resistors 106 were of value 56 Ohms. The impedance of the channels was taken from a common reference and at low frequencies, the impedances of each channel were approximately equal; at 100 kHz, the impedance of two of
20 the channels was 220 Ohms, but between the other two channels was 728 Ohms. The uneven loading of the four channels caused by the imperfections is clear.

The arrangement of Figure 12 can be used to monitor the
25 electrode array after cleaning and at intervals during use to detect any degradation.

Examples of electrode degradation in use are illustrated in Figures 13 and 14. In Figure 13, in a serpentine TWD
30 array, every fourth electrode, indicated by the arrows; has deteriorated, while the other electrodes are intact; this means that all electrodes in the same channel of a four-phase TWD system have deteriorated. This type of defect can occur if the signal applied to one of the
35 phased signals is not exactly matched to the others, e.g. it has a DC offset. The non-match may be very

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small and the defect will then develop slowly with time;
it may also be difficult to detect from external
measurements and direct monitoring of the electrode
array as described above is highly advantageous. Cell
5 debris can also be seen as indicated at D.

Figure 14 shows a serpentine TWD electrode array in
which all electrodes have deteriorated in the areas
indicated by the arrows. The electrodes in the array
10 are 8 microns wide, the inter electrode gap is 8 microns
at the narrowest point and 17 microns at the widest
point. The deterioration was the result of a large
electrical signal (4 to 12 volts) with a medium of
conductivity 200 $\mu\text{s}/\text{cm}$ placed over the electrodes and
15 can be seen to be greatest at the narrowest inter-
electrode gap where the electrical field is greatest.

Figure 15 illustrates an alternative arrangement for
sensing electrode deterioration. A linear array of
20 electrodes 144 is connected, as is conventional, by
means not shown to the signal generator to provide
signals to generate a TWD field. As usual, the
connections are made to alternate ends of the electrodes
along the array. Some electrodes, for example one in
25 twenty or one in ten, are provided with a disengageable
connection at opposite ends, i.e. the pairs of
connections 146 or the pair 148.

When a pair of connections is connected to the opposite
30 ends of an electrode 146, a measurement can be made of
the current flowing through, and therefore the impedance
of, that electrode. If the electrode is degrading, its
impedance will change, with impedance increasing for a
thinner electrode.

35 Conveniently the measurements are made on only one in

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ten or one in twenty electrodes, the assumption being that all electrodes, or all electrodes in the same channel, would degrade at a similar rate. Also, the measurements can be made while an AC electrokinetic process is running, by rapid disconnection of the signal from the electrodes, rapid measurement of electrode resistance, and rapid reconnection. The AC electrokinetic process is then subjected to minimum disruption, but electrode degradation can be monitored on a statistical basis throughout the process.

As an alternative to monitoring impedance to determine electrode deterioration, the resistance of selected electrodes can be measured to determine their temperature so that temperature variations along the array 144 can be sensed; this either assumes that there is no electrode degradation, or that electrode determination is compensated for. In an arrangement similar to that shown in Figure 9, control of temperature can be achieved.

By a variation according to known principles, the impedance or the capacitance of adjacent pairs of electrodes can be measured, to determine conductivity, or other parameters previously referred to.

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AMENDED CLAIMS

[received by the International Bureau on 4 December 2000 (04.12.00);
original claims 1 and 19 amended; remaining claims unchanged (5 pages)]

1. An electrode formation for use in an AC
electrokinetic effect cell comprising a substrate and
5 carried on the substrate
a set of a plurality of electrodes arranged for the
application of an AC electrokinetic force; and
a measurement electrode array arranged for the
measurement of an AC electrokinetic-related parameter.
- 10 2. An electrode formation according to Claim 1 in
which the set of a plurality of electrodes is
connectable to measurement means to measure the
permittivity or the electrical conductivity or the
15 temperature or the pH or the flow rate of a liquid or
the chemical content in the vicinity of the array or the
integrity of the electrodes.
- 20 3. An electrode formation according to Claim 1 in
which the measurement electrode array is connectable to
measurement means to measure the permittivity or the
electrical conductivity or the temperature or the pH or
the flow rate or the chemical content of a liquid in the
vicinity of the array.
- 25 4. An electrode formation according to Claim 1 in
which the measurement electrode array is connectable to
measurement means to detect the presence or the passage
of particles adjacent the array.
- 30 5. An electrode formation according to Claim 1, 2 or 3
in which the measurement electrode array is a single
elongated electrode and is connectable to means to
measure temperature.
- 35 6. An electrode formation according to Claim 1, 2 or 3
in which the measurement electrode array is a pair of

AMENDED SHEET (ARTICLE 19)

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spaced electrodes connectable to means to measure permittivity or electrical conductivity.

7. An electrode formation according to Claim 1, 2 or 3 in which the measurement electrode array is a pair of spaced electrodes having between them a piece of silicon, the electrodes being connectable to means to measure pH.

8. An electrode formation according to Claim 1, 2 or 3 in which the measurement electrode array is a pair of spaced electrodes, the surface of one electrode carrying a film of an inorganic salt, the electrodes being connectable to means to measure pH or chemical content.

9. An electrode formation according to Claim 1, 2 or 3 in which the measurement electrode array is an elongated electrode spaced across the direction of fluid flow, connectable to means to supply a heating current to the wire and means to sense the temperature of the wire and the fluid flow.

10. An electrode formation according to Claim 1, 2 or 4 in which the measurement electrode array is a pair of electrodes spaced across the direction of particle movement, the electrodes being connectable to means to measure impedance.

11. An electrode formation according to Claim 10 further comprising a second pair of electrodes spaced across the direction of particle movement and spaced in the direction of flow from the first pair, both pairs being connectable to means to measure the speed of the particle between the pairs of electrodes.

12. An electrode formation according to Claim 10 or 11 connectable to means to indicate cessation of particle

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flow.

13. An electrode formation according to Claim 10 further comprising two or more further arrays of TWD electrodes diverging from an output end of said set of electrodes for application of a TWD force, and means to energise one of said further arrays in accordance with a sensed property of a particle.
14. An electrode formation according to any preceding Claim in which the set of a plurality of electrodes and the measurement electrode array are arranged on the substrate so as to overlap, there being an insulating film between the set and the array.
15. An AC electrokinetic effect system comprising an electrode formation according to any preceding Claim, a second substrate spaced from the first, signal supply means to apply an electrical signal to the set of a plurality of electrodes to generate an AC electrokinetic force, and measuring means to measure an AC electrokinetic-related parameter connected to the measurement electrode array.
16. A system according to Claim 15 further comprising adjustment means to adjust said AC electrokinetic-related parameter in accordance with the value sensed by the measuring means.
17. A system according to Claim 16 in which the AC electrokinetic-related parameter is temperature and the adjustment means is a Peltier cooling device.
18. A system according to Claim 16 in which the AC electrokinetic-related parameter is chemical composition, and the adjustment means is selective application of a protein or of a protein and a protein-

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binding ligand.

19. An AC electrokinetic effect electrode test system comprising an array of electrodes carried on a substrate arrangement for the application of a DEP or TWD or electrorotation force;

and connected to the electrodes, signal supply means to apply at least one electrical signal to generate an AC electrokinetic force; and measuring means to measure an AC electrokinetic-related parameter or the integrity of the electrodes.

20. A test system according to Claim 19 in which the measuring means is arranged to measure the impedance of at least one electrode.

21. A test system according to Claim 20 in which the measuring means is intermittently connectable to opposite ends of a proportion of the electrodes in the array.

22. A test system according to Claim 19 in which the measuring means is arranged to measure the capacitance or impedance of a part of the length of two adjacent electrode.

23. A method of measuring an AC electrokinetic-related parameter of a suspension of particles in a liquid comprises applying an AC electrokinetic force to said suspension by means of an array of electrodes, and measuring said parameter by connection of said array to electrical measuring means.

24. A method of maintaining conditions for viability of a type of particle during application of an AC electrokinetic process comprises suspending the particle in a fluid; applying to the suspension an AC

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electrokinetic force; sensing an AC electrokinetic-related parameter upon which viability is dependent; and adjusting said parameter to maintain viability.

5 25. A method according to Claim 24 to which said parameter is temperature.

26. A method according to Claim 24 to which said parameter is pH.

10

27. A method of assisting an AC electrokinetic process in the separation of two types of particles in a suspension comprises applying to the suspension an AC electrokinetic force; sensing an AC electrokinetic-related parameter of the suspension; controlling said parameter so that one type of particle remains viable, while the other type of particle is subject to a deleterious effect; and applying an AC electrokinetic force whereby the particle types are separated.

15

28. A method according to Claim 27 in which said parameter is temperature or pH and is controlled so that said other type of particle dies or is denatured.

25 29. An electrode formation as hereinbefore described with reference to any one of Figures 1, 2, 4, 5, 6, 7 or 8 of the accompanying drawings.

30 30. An AC electrokinetic system as hereinbefore described with reference to Figure 3 or 9 of the accompanying drawings.

35 31. An AC electrokinetic test system as hereinbefore described with reference to Figure 12 or 15 of the accompanying drawings.

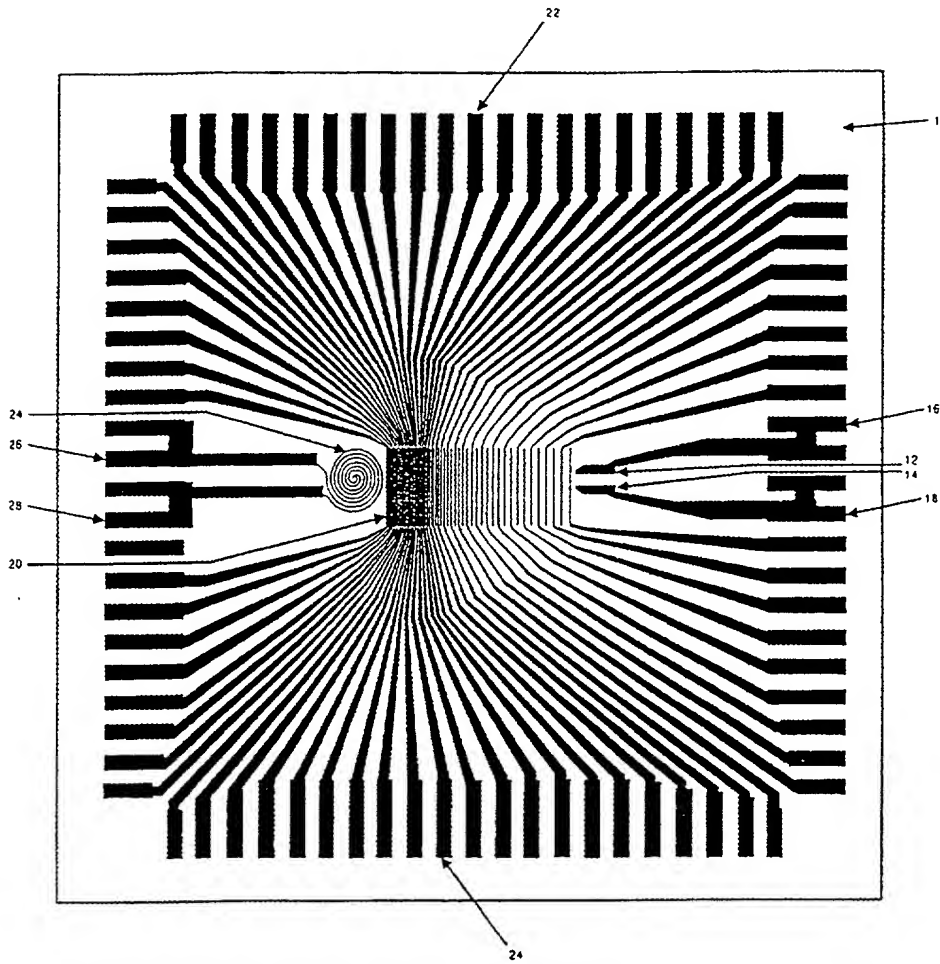


Fig. 1a

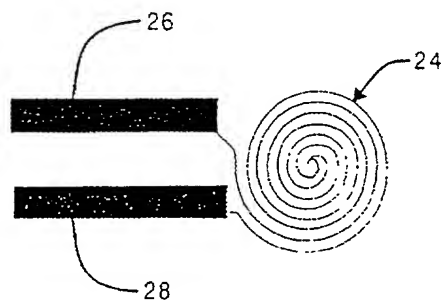


Fig. 1b

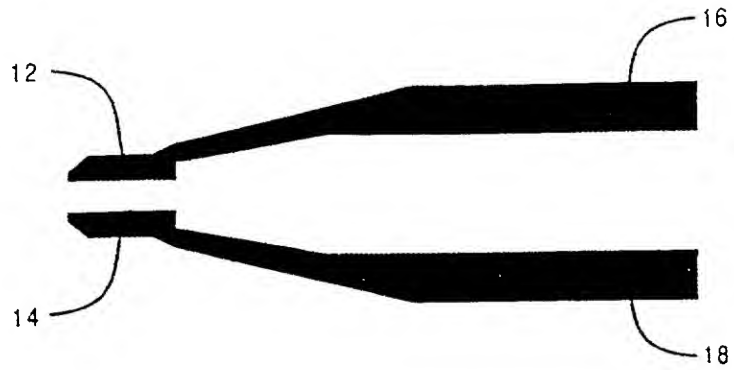


Fig. 1c

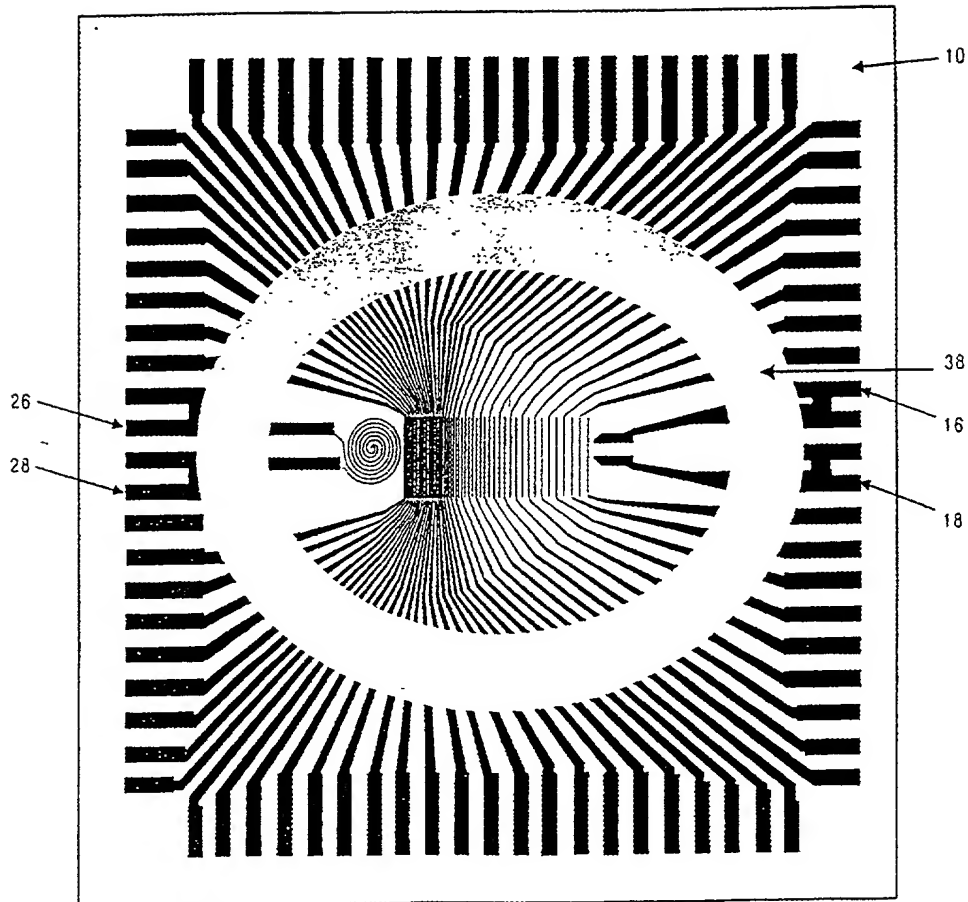


Fig. 2

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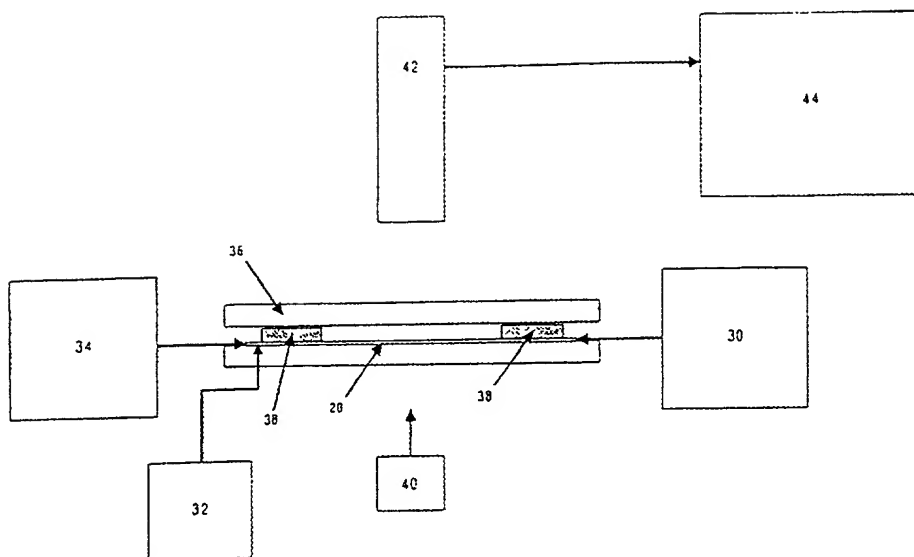


Fig. 3

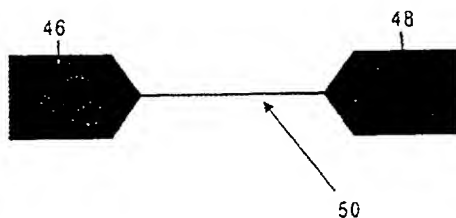


Fig. 4

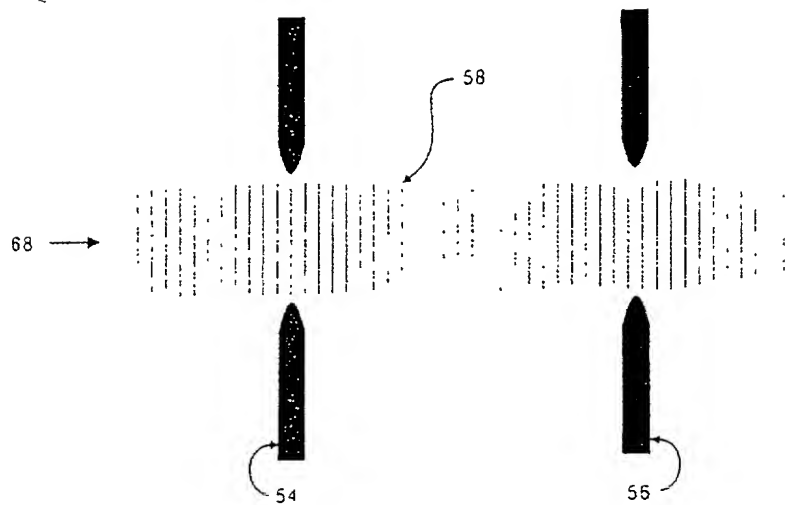


Fig. 5

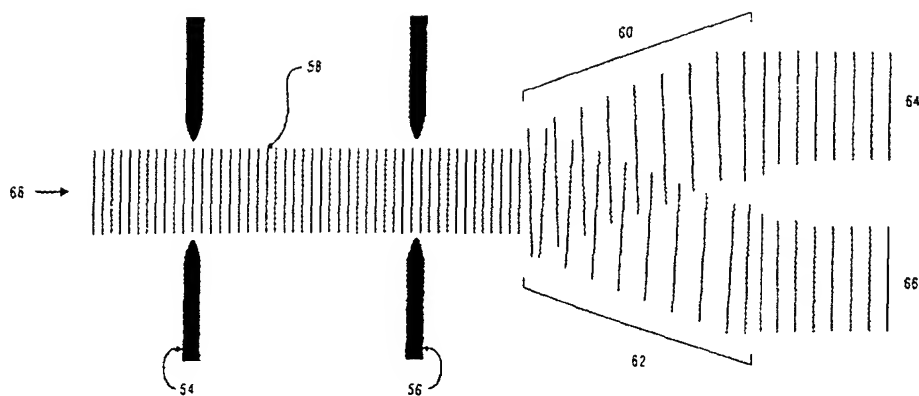


Fig. 6

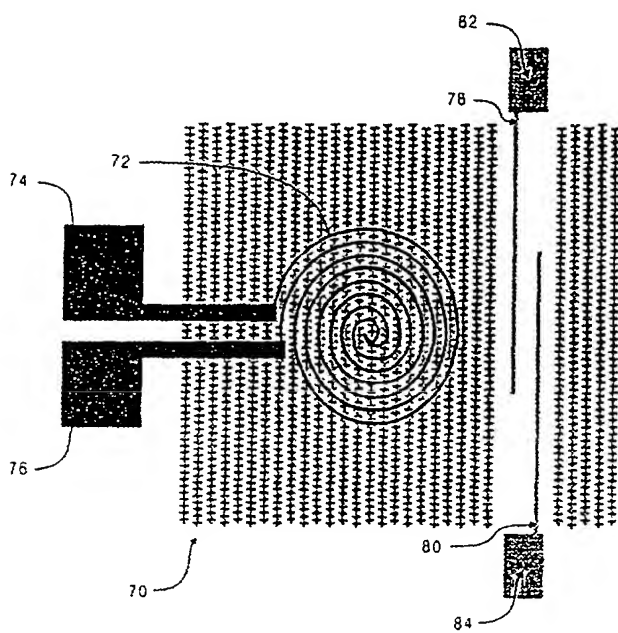


Fig. 7

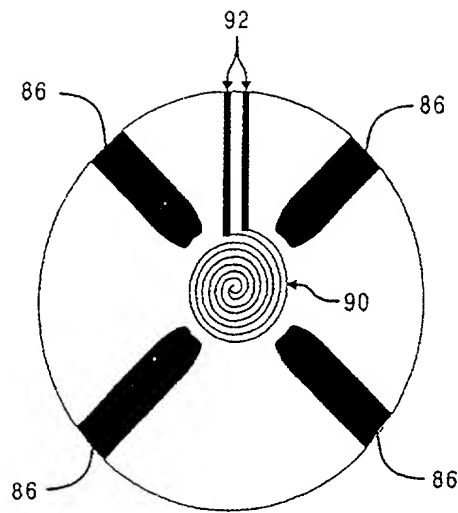


Fig. 8

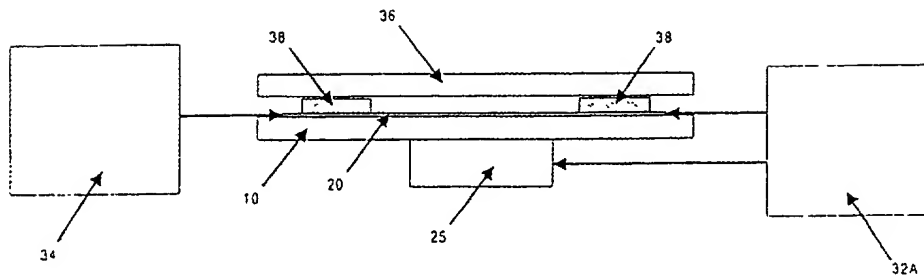


Fig. 9

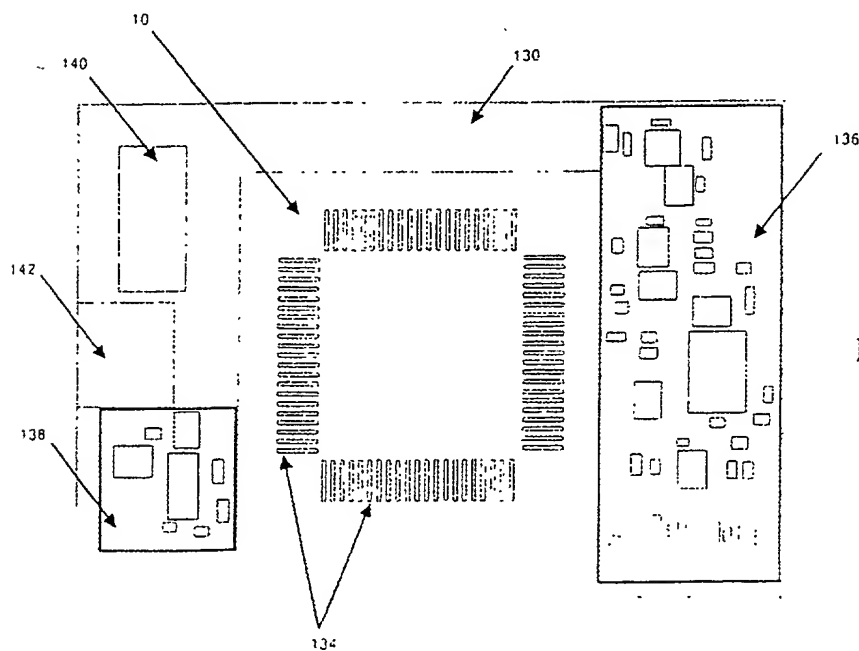


Fig. 10

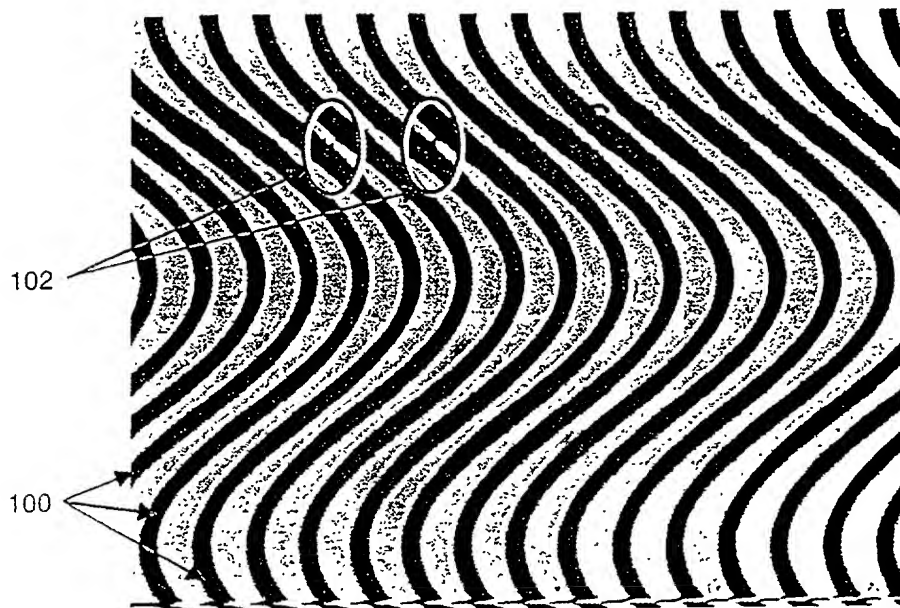


Fig. 11

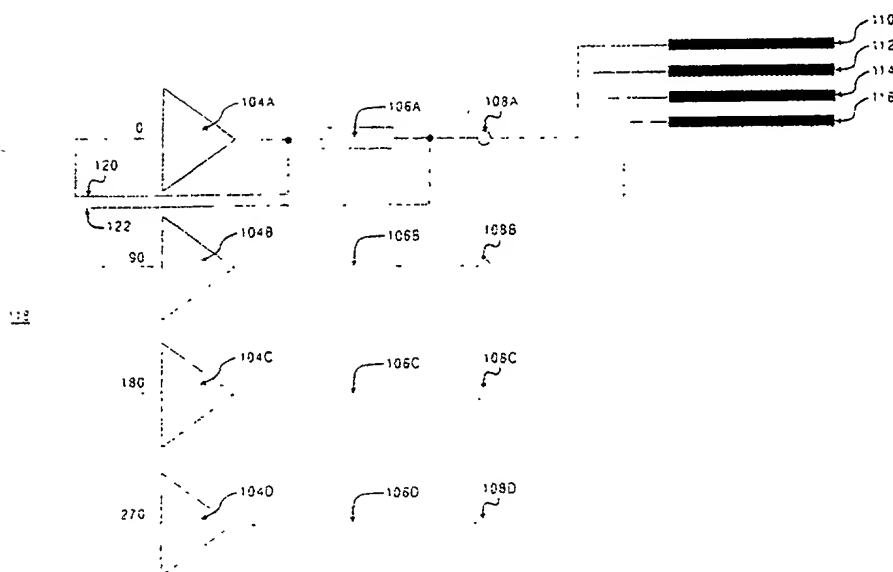


Fig. 12

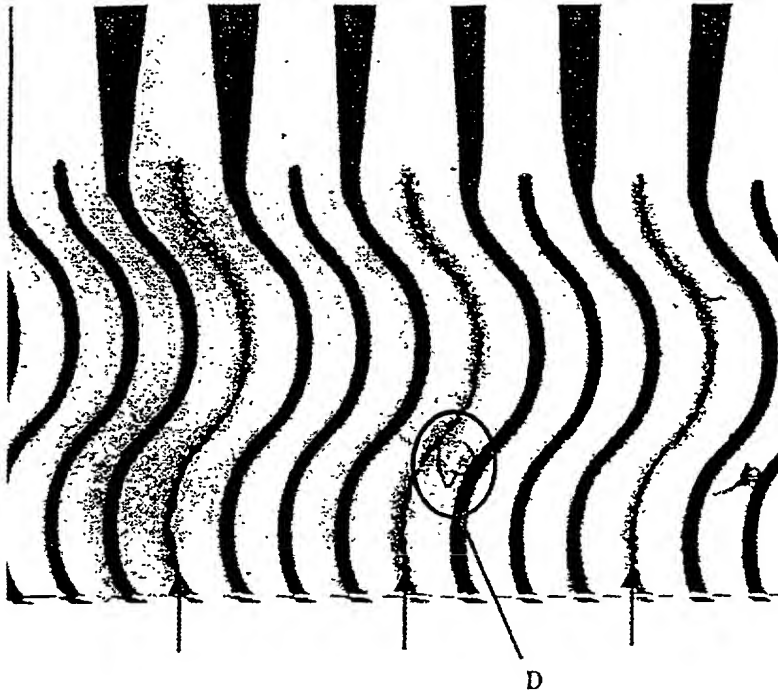


Fig. 13

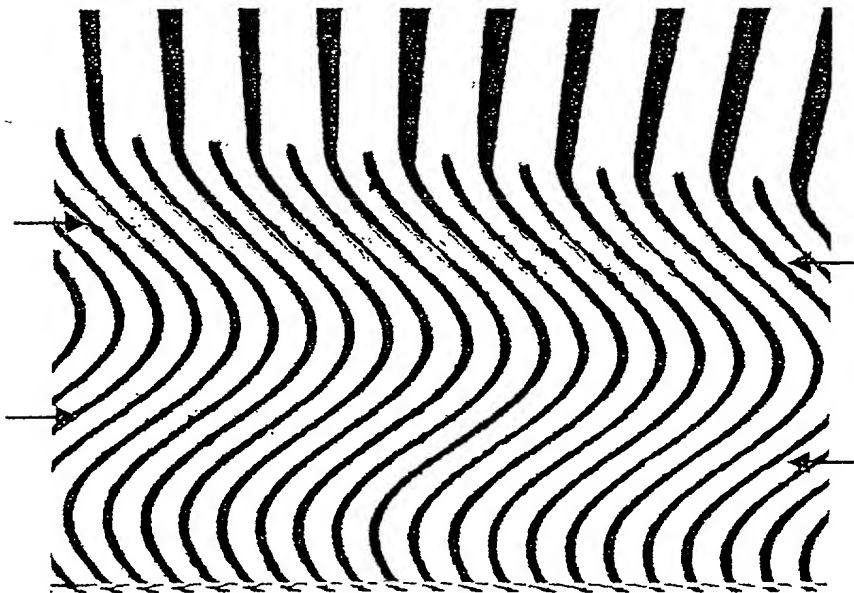


Fig. 14

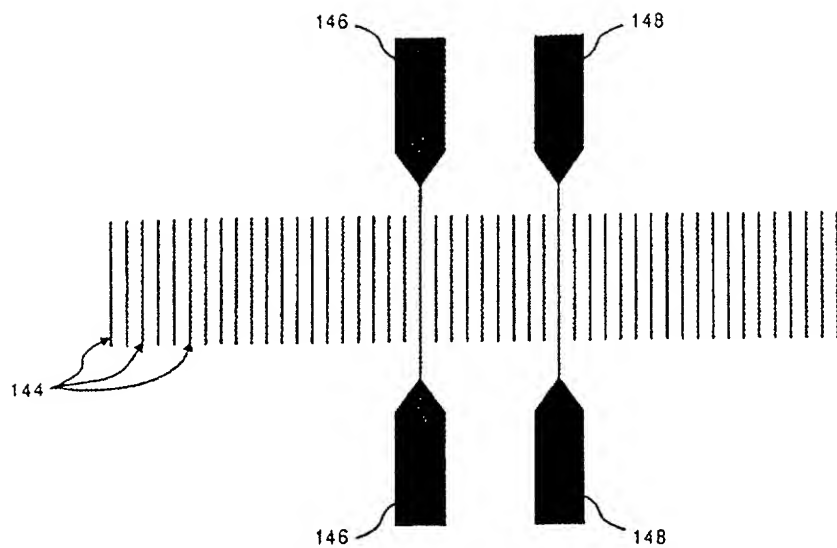


Fig. 15

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Fig. 16

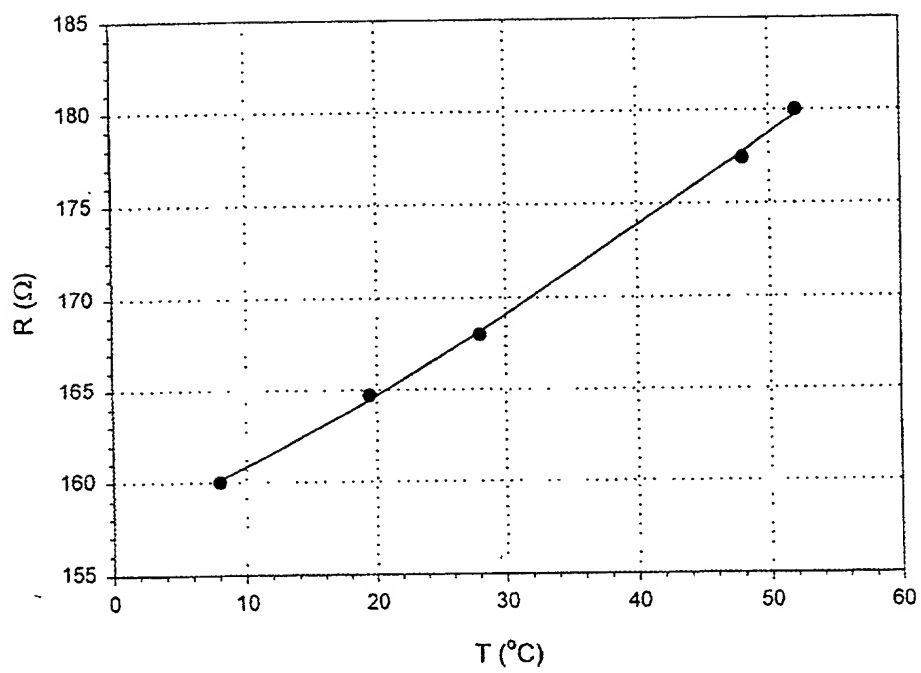
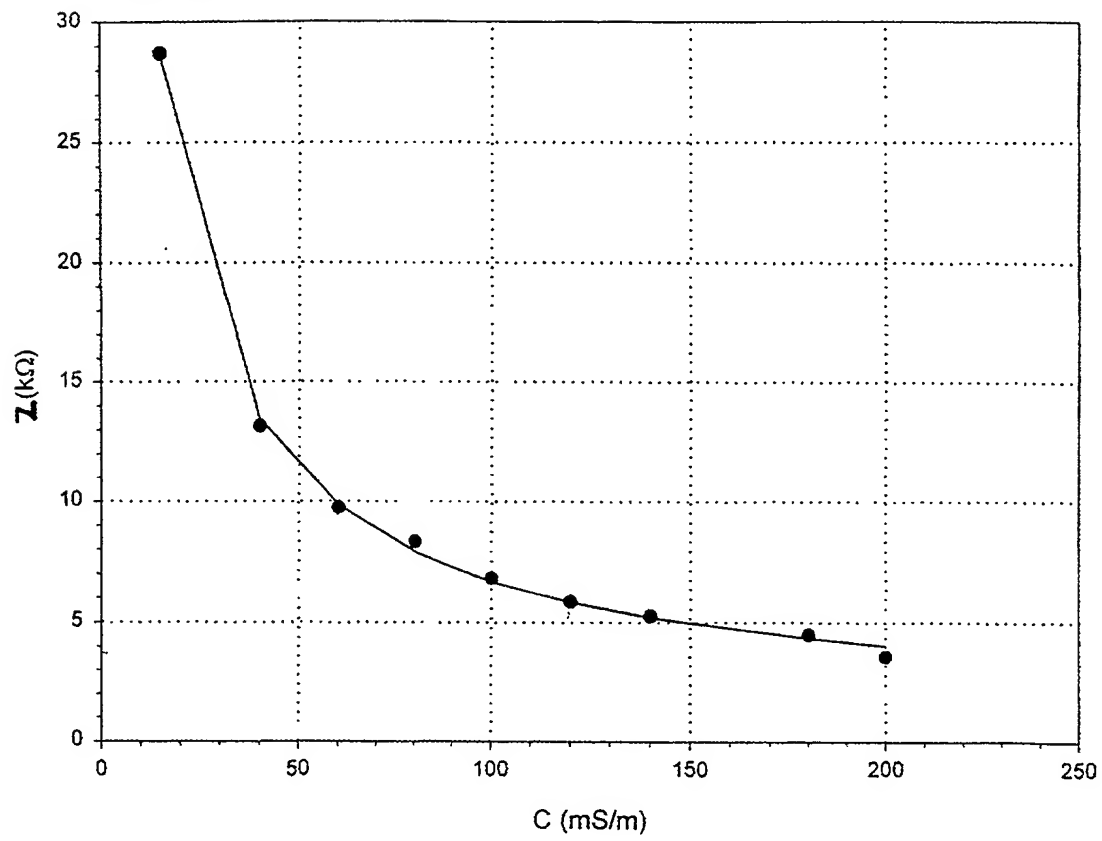


Fig. 17



COMBINED DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

Docket No. 5625

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ELECTRODES FOR GENERATING AND ANALYZING DIELECTROPHORESIS, the specification of which is attached hereto unless the following box is checked:

[X] was filed on July 20, 2000 as United States Application Number or PCT International Application Number PCT/GB00/02801 and was amended on December 4, 2000 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)	Priority Claimed
<u>PCT/GB00/02801</u> <u>PCT</u> <u>20 July 2000</u> (Number) (Country) (Day/Month/Year Filed)	Yes [X] No []
<u>9916851.0</u> <u>Great Britain</u> <u>20 July 1999</u> (Number) (Country) (Day/Month/Year Filed)	Yes [X] No []
_____ (Number) (Country) (Day/Month/Year Filed)	Yes [] No []

COMBINED DECLARATION & POWER OF ATTORNEY

Docket No. 5625

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below,

(Application Number)

(Filing Date)

(Application Number)

(Filing Date)

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

(Application No.)

(Filing Date)

(Status-patented, pending, abandoned)

(Application No.)

(Filing Date)

(Status-patented, pending, abandoned)

I (we) hereby appoint the following attorney with full power of substitution to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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Mary J. Breiner at (703) 684-6885

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full Name of Third Joint Inventor, if any

(given name, family name) _____

Inventor's Signature _____

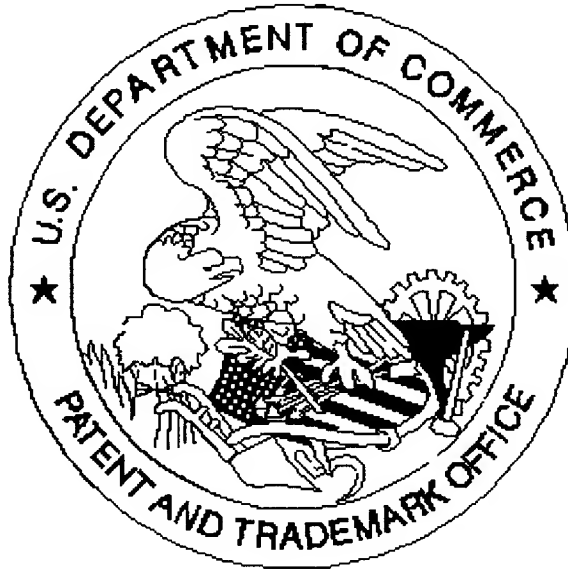
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